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Impact of ACE2 and TMPRSS2 Expression in Patients With Preeclampsia

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Abstract. *Background/Aim: As maternal morbidity and mortality during pregnancy have increased during the COVID-19 pandemic, studies on pregnancy-related complications from SARS-CoV-2 infection are being actively conducted. Considering that pregnant women with COVID-19 may develop a preeclampsia (PE)-like syndrome, it is necessary to differentiate it from PE because true PE can result in an unfavorable perinatal outcome during a hasty delivery. Materials and Methods: We investigated the protein expression of transmembrane serine protease 2 (TMPRSS2) and angiotensin-converting enzyme 2 (ACE2) in placental samples from 42 normotensive (n=9) and PE (n=33) patients without SARS-CoV-2 infection. We isolated placental trophoblast cells from normotensive and PE patients without evidence of SARS-CoV-2 infection to determine the mRNA and protein expression levels of TMPRSS2 and ACE2. Results: High ACE2 cytoplasmic expression in extravillous trophoblasts (EVTs) was correlated with lower fibrin deposition (p=0.017). In comparison with high nuclear TMPRSS2 expression, low nuclearTMPRSS2 expression in endothelial cells (ECs) was positively correlated with PE (p=0.005), significantly higher systolic blood pressure (p=0.006), and higher urine protein-to-creatinine ratio (p=0.022). In contrast, high cytoplasmic TMPRSS2 expression in fibroblasts (FBs) was correlated with higher urine protein-to-creatinine ratio (p=0.018). Trophoblast cells extracted from PE placental tissue showed lower mRNA levels for both ACE2 and TMPRSS2. Conclusion: TMPRSS2 nuclear expression in ECs and cytoplasmic expression in FBs of the placenta may be related to a trophoblast-independent PE mechanism, and TMPRSS2 could be a new biomarker to discriminate actual PE from a PE-like syndrome associated with COVID-19.*

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Coronavirus disease 19 (COVID-19) is continuously causing loss of life and deterioration of health worldwide due to the emergence of various variants since the pandemic outbreak in 2019. Accordingly, research on morbidity, prevention, and treatment has been actively conducted. The obtained findings have shown that pregnant women are susceptible to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (1), and it has been reported that infection increases the risk of adverse pregnancy outcomes, premature birth, stillbirth, and preeclampsia (PE) (2-4).

PE is part of the spectrum of pregnancy-related hypertensive disorders and results in decreased multi-organ perfusion. At the mild end of the spectrum is gestational hypertension, with the most severe form of the hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome at the other end. Currently, the only effective cure for controlling maternal and fetal PE complications is optimally timed birth. More than 15% of all preterm births are associated with pregnancy-related hypertensive disorders (5). Although the exact mechanism and cause of PE have not

Table I. *Clinicopathological data of 42 patients.*

		Normotensive group n=9	Preeclampsia group n=33	*p*-Value
Age, years	Mean±SD	33.6±4.2	34.4±5.1	0.668
Gestational age, weeks		32.0±4.8	31.7±5.1	0.881
Systolic blood pressure, mmHg		139.3±10.7	168.2±15.8	**<0.001**
Diastolic blood pressure, mmHg		88.3±8.2	105.0±12.3	**0.001**
Urine protein-to-creatinine ratio		0.2±0.1	2.5±3.1	**0.001**
Increased syncytial knots, n (%)		7 (77.8%)	31 (93.9%)	0.410
Increased intervillous fibrin, n (%)		0 (0.0%)	6 (18.2%)	0.398
Inflammation, n (%)		2 (22.2%)	1 (3.0%)	0.211
IVT.ACE2.expression, n (%)	Low	3 (33.3%)	12 (36.4%)	1.000
	High	6 (66.7%)	21 (63.6%)	
EVT.ACE2.expression, n (%)	Low	2 (22.2%)	7 (21.2%)	1.000
	High	7 (77.8%)	26 (78.8%)	
EC.ACE2.expression, n (%)	Low	7 (77.8%)	32 (97.0%)	0.211
	High	2 (22.2%)	1 (3.0%)	
IVT.TMPRSS2.expression, n (%)	Low	1 (11.1%)	13 (39.4%)	0.231
	High	8 (88.9%)	20 (60.6%)	
EVT.TMPRSS2.expression, n (%)	Low	6 (66.7%)	18 (54.5%)	0.786
	High	3 (33.3%)	15 (45.5%)	
EC TMPRSS2 expression, n (%)	Low	5 (55.6%)	32 (97.0%)	**0.005**
	High	4 (44.4%)	1 (3.0%)	
FB TMPRSS2 expression, n (%)	Low	0 (0.0%)	4 (12.1%)	0.647
	High	9 (100.0%)	29 (87.9%)	

SD: Standard deviation; IVT: intravillous trophoblast; EVT: extravillous trophoblast; EC: endothelial cells; FB: fibroblast. Bolded values represent statistical significance.

been identified, it is widely known that incomplete vascular transformation in PE causes high blood pressure, poor perfusion, and villous malformation (6).

Cell entry of SARS-CoV-2 depends on transmembrane serine protease 2 (TMPRSS2) and angiotensin-converting enzyme 2 (ACE2), and blockage of entry by a protease inhibitor has been clinically proven (7). ACE2 is a protein located on the cell surface to which the SARS-CoV-2 spike protein may bind to infected cells. Previously, researchers have identified entry of SARS-CoV-2 through ACE2 based on down-regulation of membrane-bound ACE2 in the placenta. In particular, SARS-CoV-2 infection has been associated with a decrease in ACE2 expression in placental cells including trophoblasts and endothelial cells (ECs) of the mother and baby (7). Down-regulated ACE2 impairs the function of the renin-angiotensin system, leading to hemodynamic consequences such as PE (8) and increased production of PE markers, including soluble fms-like tyrosine kinase-1 (sFlt1) (9). TMPRSS2 has been found to be involved in S protein priming in SARS-CoV-2 infection, and its inhibitor has been clinically approved and is commercially available (7). In addition, expression of TMPRSS2 has been found to be positively correlated with type 2 cytokines and is increased in airway epithelial cells in allergic rhinitis and type 2 asthma (10).

Some studies have reported that pregnant women infected with COVID-19 may develop a PE-like syndrome that is not a placental complication, which can be distinguished from actual PE (11, 12). Hecht *et al*. examined the histopathology of COVID-19-exposed placentas and expression of ACE2 and TMPRSS2 by immunohistochemical staining and found that SARS-CoV-2 infection of the placenta was not associated with a specific placental histopathology (13). In particular, there was a weakly positive cytoplasmic TMPRSS2 expression in the villous endothelial cells in rare cases of the SARS-COV-2 infected placentas. Only one case had very weak and patchy positive membranous TMPRSS2 expression in the syncytiotrophoblast cells. Most cases were negative for TMPRSS2 expression. In this study, we investigated the expression of TMPRSS2 and ACE2 in placental samples from normotensive and PE patients without SARS-CoV-2 infection. We determined the potential role of TMPRSS2 expression to differentiate true PE from PE-like syndrome associated with SARS-COV-2 infection.

Materials and Methods

A total of 42 patients who gave birth between January 2021 and December 2021 at Gyeongsang National University Changwon Hospital (Changwon, Republic of Korea) were enrolled in the study.

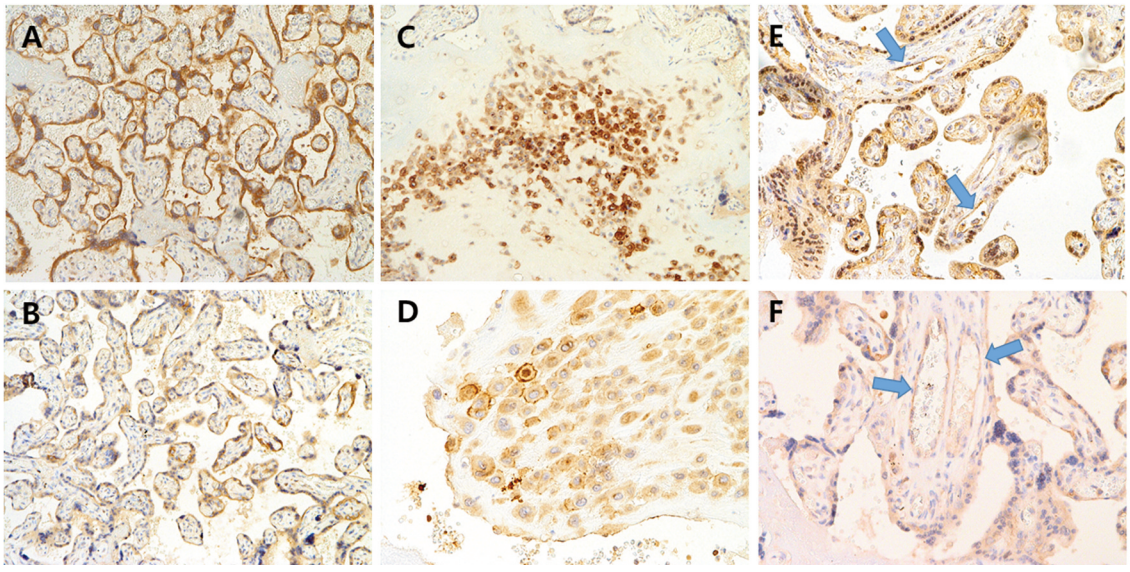

Figure 1. *ACE2 expression in human PE placental tissue. (A) High cytoplasmic and membranous expression in IVTs with an intensity of 4. (B) Low cytoplasmic and membranous expression in IVTs with an intensity of 1. (C) High cytoplasmic and membranous expression in EVTs with an intensity of 4. (D) Low cytoplasmic and membranous expression in EVTs with an intensity of 1. (E) High cytoplasmic and membranous expression in ECs (arrow) with an intensity of 3. (F) Low cytoplasmic and membranous expression in ECs (arrow) with an intensity of 1. PE: Preeclampsia; IVT: intravillous trophoblast; EVT: extravillous trophoblast; EC: endothelial cells.*

Of the 42 pregnant patients, 33 patients with PE belonged to the PE group (study group), and 9 gestational age-matched normotensive patients with normal blood pressure and without proteinuria belonged to the normotensive group. The PE group included gestational hypertension, PE, and severe PE according to the American College of Obstetricians and Gynecologists (ACOG) diagnostic criteria (14) and HELLP according to the Tennessee Classification System (15). The normotensive group included normal full-term delivery without any problems and preterm delivery; the reasons for preterm delivery were cervical insufficiency, preterm labor, and premature rupture of the amniotic membrane. At the hospital, a nasopharyngeal swab for SARS-CoV-2 detection by PCR was performed on all patients at admission for delivery and repeatedly every 3 days thereafter. Only patients who were SARS-CoV-2-negative were included in the study. Representative hematoxylin and eosin-stained slides of selected placental samples were examined. Clinical data were obtained by reviewing the electronic medical records, such as age, gestational age, and factors affecting the degree of PE, including blood pressure and amount of proteinuria. Representative pathological features including syncytial knots, fibrin deposition, and inflammation (necrotizing chorioamnionitis) were recorded. Clinicopathological data were compared between patients with and without PE and further evaluated for correlation with ACE2 and TMPRSS2 expression (Table I).

Ethics. This study was approved by the Institutional Review Board of Gyeongsang National University Changwon Hospital (serial number: GNUCH 22-08-015).

Immunohistochemistry of human placenta tissue samples. Four representative hematoxylin and eosin-stained glass slides containing umbilical cord (one slide), chorioamniotic membrane (one slide), and chorionic disc (two slides) samples were examined. Immunohistochemical staining was performed using an automated immunostainer (BenchMark ULTRA; Ventana Medical Systems Inc., Tucson, AZ, USA) with the following monoclonal antibodies: ACE2 (#ab15348; Abcam, Cambridge, UK) with 1:500 dilution and TMPRSS2 (#ab92323; Abcam) with 1:1000 dilution. The immunohistochemical staining patterns of ACE2 and TMPRSS2 were evaluated for the two slides containing chorionic disc samples. ACE2 and TMPRSS2 expression levels were evaluated in three different cells including intravillous trophoblasts (IVTs) (syncytiotrophoblasts and cytotrophoblasts), extravillous trophoblasts (EVTs) (trophoblasts located in the cell island and septa), and ECs. In addition, fibroblasts (FBs) on villi were evaluated only for TMPRSS2 expression. The intensity of the stained tumor cells was scored as follows: unstained: 0; weakly stained: 1; moderately stained: 2; strongly stained: 3; extensively and strongly stained: 4. Representative microscopic images are presented in Figure 1 and Figure 2. ACE2 assessment included (i) cytoplasmic and membranous expression in IVTs with an intensity ranging from 0 to +4 (Figure 1A: high, Figure 1B: low), (ii) cytoplasmic and membranous expression in EVTs with an intensity ranging from +1 to +4 (Figure 1C: high, Figure 1D: low), and (iii) cytoplasmic and membranous expression in ECs with an intensity ranging from 0 to +3 (Figure 1E: high, Figure 1F: low). TMPRSS2 assessment included (i) nuclear expression in IVTs with an intensity ranging from 0 to +4 (Figure 2A: high, Figure 2B: low), (ii) cytoplasmic and membranous expression in EVTs with an intensity ranging from 0 to +4 (Figure 2C: high, Figure 2D: low), (iii) nuclear expression in ECs with an intensity ranging from 0 to +3 (Figure 2E: high, Figure 2F: low), (iv) and cytoplasmic expression in FBs with an intensity ranging from +1 to +4 (Figure 2G: high, Figure 2H: low).

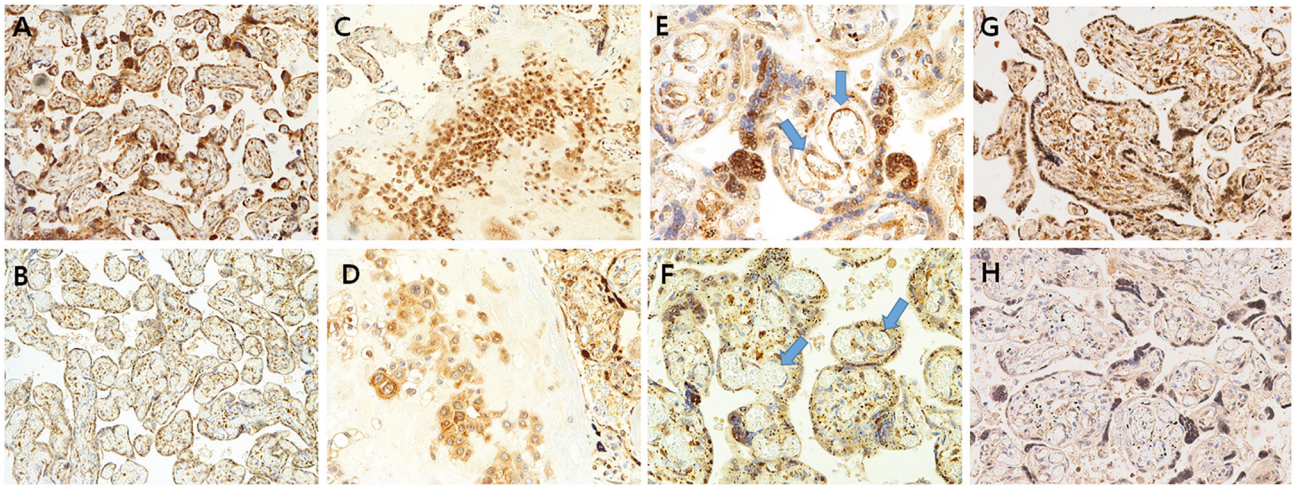

Figure 2. *Evaluation of TMPRSS2 expression in human PE placental tissue using immunohistochemistry. (A) High nuclear expression in IVTs with an intensity of 4. (B) Low nuclear expression in IVTs with an intensity of 1. (C) High cytoplasmic and membranous expression in EVTs with an intensity of 4. (D) Low cytoplasmic and membranous expression in EVTs with an intensity of 1. (E) High nuclear expression in ECs (arrow) with an intensity of 3. (F) Low nuclear expression in ECs (arrow) with an intensity of 1 (G). High cytoplasmic expression in FBs with an intensity of 4. (H) Low cytoplasmic expression in FBs with an intensity of 1. PE: Preeclampsia; IVT: intravillous trophoblast; EVT: extravillous trophoblast; EC: endothelial cells; FB: fibroblast.*

Cell lines. Primary human trophoblast cells were isolated from placental samples using Percoll gradient (16). Isolated cells were cultured in DMEM (#11995-065; Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; #F0900; GenDEPOT, Katy, TX, USA), 1% penicillin-streptomycin (#30-002-CI; Corning Inc., Corning, NY, USA), and primocin (ant-pm-2; Invitrogen, Waltham, MA, USA) at 37˚C with 5% CO2.

Semi-quantitative PCR. Total RNA was extracted using the TRIzol reagent (Qiagen, Hilden, Germany). Quantification of total RNA was performed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and 1 μg of total RNA was reverse transcribed to cDNA using the Maxime RT PreMix Kit (#25081; iNtRON, Seongnam, Republic of Korea). An equal amount of synthesized cDNA was used to perform semi-quantitative PCR with the Maxime PCR PreMix Kit (#25025; iNtRON). Quantification was performed using GAPDH as a reference gene. The ACE2 (#P123756) and TMPRSS2 (#P146872) primers were purchased from Bioneer (Daejeon, Republic of Korea). The GAPDH primer sequences were as follows: forward, 5'-GTC CAC CAC CCT GTT GCT GTA G-3'; reverse, 5'-CAA GGT CAT CCA TGA CAA CTT TG-3'.

Western blotting. Proteins were extracted using RIPA lysis buffer (#89900; Thermo Fisher Scientific) with a protease inhibitor cocktail (#78430; Thermo Fisher Scientific). The total protein concentration of each cell lysate was measured using the Bradford method with bovine serum albumin as a standard. Equal amounts of protein lysates (50 μg) were loaded on a denaturing polyacrylamide gel and transferred to a nitrocellulose membrane. The primary antibodies used for immunoblotting were TMPRSS2 (#ab92323; Abcam) and GAPDH (#ab8245; Abcam) with horseradish peroxidase-conjugated secondary antibodies, and immunoreactive bands were developed by enhanced chemiluminescence reaction (#32109; Thermo Fisher Scientific). The digital chemiluminescence images were taken by Fusion Solo (Vilber, Collégien, France).

Statistical analysis. The relationship between ACE2 and TMPRSS2 expression and pathological or clinical data was evaluated by the Pearson's Chi-square test and Fisher's exact test. A *p*-value less than 0.05 was considered statistically significant. Statistical analyses were performed using R 4.0.3.

Results

Clinicopathological characteristics of patients with preeclampsia. A total of 42 patients were enrolled in the study. The clinical and pathological variables of patients were analyzed according to the presence of PE and levels of ACE2 and TMPRSS2 expression (Table I). The mean age of the patients was 34.2±4.9 years, and the mean gestational age was 31.8±5.0 weeks. There were 9 patients in the normotensive group, and the PE group included patients with gestational hypertension (n=3), PE (n=9), severe PE (n=9), and HELLP syndrome (n=4). All patients enrolled in the study were Korean adults. In addition, the selection of the study cohort was unbiased in terms of clinical grading of PE, fibrin deposition, inflammation, EVT ACE2 expression, EC TMPRSS2 expression, IVT TMPRSS2 expression, and FB TMPRSS2 expression, as shown in Table I. There was no difference in age or gestational age between the normotensive group and the PE group, and a significantly lower expression of TMPRSS2 in ECs was observed in the PE group (p=0.005).

Table II. *Correlation of ACE2 expression with clinicopathological factors (n=42).*

	EVT.ACE2.expression		
	Low (1) (n=9)	High (2-4) (n=33)	*p*-Value
Normotensive group, n (%)	2 (22.2%)	7 (21.2%)	1.000
Preeclampsia group, n (%)	7 (77.8%)	26 (78.8%)	
Systolic blood pressure, mmHg	166.5±29.4	158.7±15.5	0.493
Diastolic blood pressure, mmHg	103.9±20.5	99.6±10.9	0.584
Urine protein-to-creatinine ratio	1.2±1.6	2.1±3.1	0.428
Increased syncytial knots, n (%)	9 (100.0%)	29 (87.9%)	**0.017**
Increased intervillous fibrin, n (%)	4 (44.4%)	2 (6.1%)	**0.017**
Inflammation, n (%)	1 (11.1%)	2 (6.1%)	1.000

EVT: Extravillous trophoblasts. Bold values represent statistical significance.

Table III. *Correlation of TMPRSS2 expression with clinicopathological factors (n=42).*

	TMPRSS2					
	EC			FB		
	Low (0-2)	High (3-4)	*p*-Value	Low (0-2)	High (3-4)	*p*-Value
Normotensive group, n (%)	5 (13.5%)	4 (80.0%)	**0.005**	0 (0.0%)	9 (23.7%)	0.647
Preeclampsia group, n (%)	32 (86.5%)	1 (20.0%)		4 (100.0%)	29 (76.3%)	
Systolic blood pressure, mmHg	164.2±18.1	139.4±12.6	**0.006**	161.3±9.6	160.5±20.2	0.944
Diastolic blood pressure, mmHg	102.1±13.9	92.0±6.7	0.125	102.7±8.1	100.4±14.0	0.785
Urine protein-to-creatinine ratio	2.2±3.0	0.5±0.8	**0.022**	0.6±0.1	2.0±2.9	**0.018**
Increased syncytial knots, n (%)	34 (91.9%)	4 (80.0%)	0.969	4 (100.0%)	34 (89.5%)	1.000
Increased intervillous fibrin, n (%)	6 (16.2%)	0 (0.0%)	0.770	0 (0.0%)	6 (15.8%)	0.915
Inflammation, n (%)	2 (5.4%)	1 (20.0%)	0.792	1 (25.0%)	2 (5.3%)	0.662

EC: Endothelial cells; FB: fibroblasts. Bold values represent statistical significance.

TMPRSS2 nuclear expression in ECs was significantly correlated with preeclampsia. ACE2 expression in EVTs was significantly correlated with fibrin deposition in PE placental tissue (Table II). High cytoplasmic and membranous ACE2 expression in EVTs was correlated with lower fibrin deposition (p=0.017). ACE2 expression did not show any correlation with PE grading; however, as fibrin deposition is one of the factors associated with PE, as demonstrated in our previous study (17), ACE2 expression in trophoblasts may be indirectly related to PE.

The results for TMPRSS2 expression are shown in Table III. TMPRSS2 nuclear expression in ECs was significantly correlated with PE. Low nuclear TMPRSS2 expression in ECs was positively correlated with PE (p=0.005), significantly higher systolic blood pressure (164.2±18.1 *vs*. 139.4±12.6 mmHg, p=0.006), and higher urine protein-to-creatinine ratio (2.2±3.0 *vs*. 0.5±0.8, p=0.022) compared to high nuclear TMPRSS2 expression. In contrast, high cytoplasmic TMPRSS2 expression in FBs was correlated with higher urine protein-to-creatinine ratio (2.0±2.9 *vs*. 0.6±0.1, p=0.018).

Trophoblast cells of preeclampsia placental tissue did not show significant difference in TMPRSS2 protein expression. The mRNA levels of *ACE2* and *TMPRSS2* were estimated from total mRNA extracted from normotensive and PE human placental trophoblast cells by semi-quantitative PCR. Quantification was performed using GAPDH as a reference gene. Trophoblast cells extracted from PE placental tissue showed lower mRNA levels compared to the normal placental tissue for both ACE2 (0.25 *vs*. 0.55) and TMPRSS2 (0.11 *vs*. 0.23) (Figure 3). Western blotting was performed to determine the protein levels of TMPRSS2 in normotensive and PE human placental trophoblast cells. Trophoblast cells extracted from PE placental tissue did not show significant differences from those extracted from normotensive placental tissue in terms of TMPRSS2 protein expression (Figure 4).

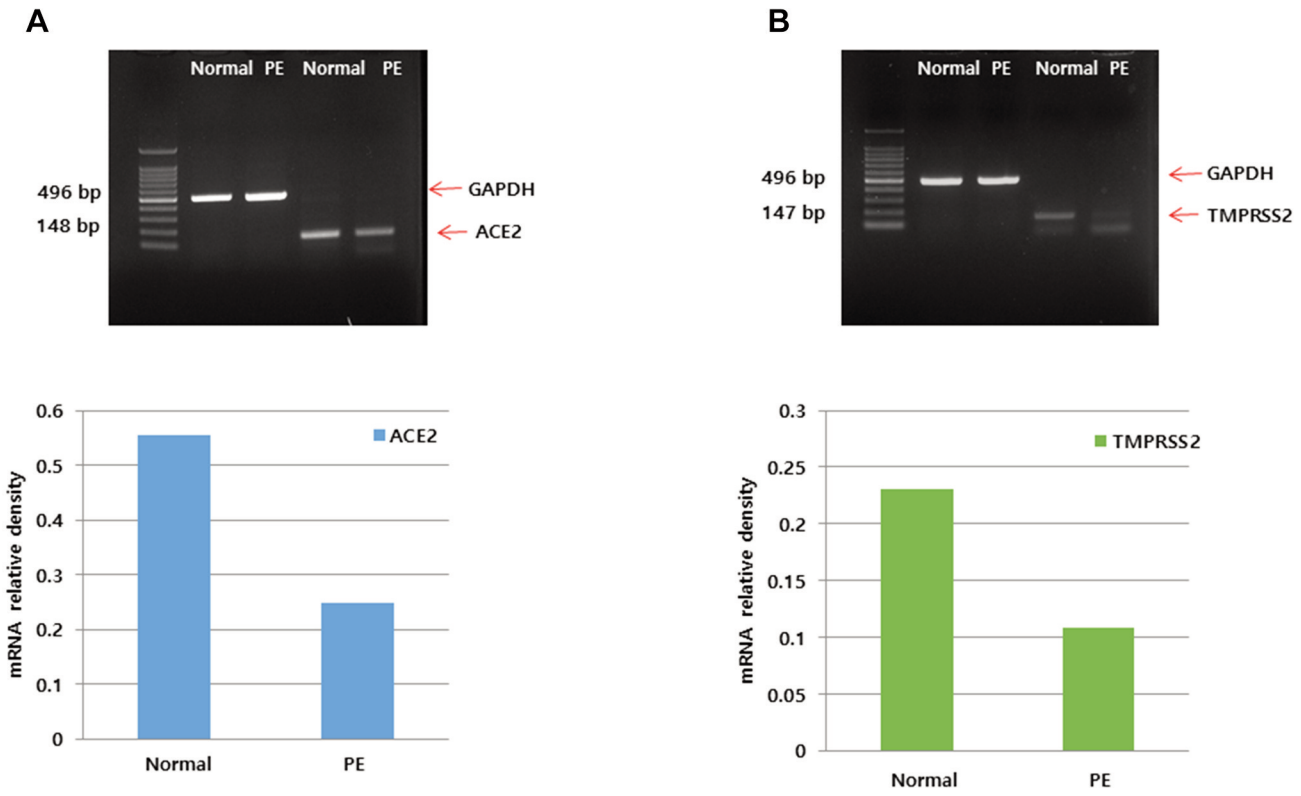

Figure 3. *ACE2 and TMPRSS2 mRNA expression in human placental trophoblast cells. (A) The relative mRNA density of ACE2 was lower in PE trophoblast cells compared to normal controls (0.25 vs. 0.55). (B) The relative mRNA density of TMPRSS2 was lower in PE trophoblast cells compared normal controls (0.11 vs. 0.23). PE: Preeclampsia.*

Discussion

In this study, TMPRSS2 and ACE2 expression levels were examined in PE placental tissue and trophoblast cells of patients without evidence of SARS-Cov-2 infection. This is the first study to evaluate the expression of these two markers in human placental tissue from patients with PE. The expression of each marker was examined for various cells constituting the placenta, including IVTs, EVTs, ECs, and FBs. Low ACE2 expression in EVTs was correlated with lower fibrin deposition supported by statistical significance. Low TMPRSS2 expression in ECs showed a correlation with PE with statistical significance. As expected, the low nuclear expression of TMPRSS2 in ECs was correlated with higher blood pressure and higher urine protein-to-creatinine ratio. On the other hand, the higher the TMPRSS2 expression in FBs, the higher the urine protein-to-creatinine ratio. The mRNA expression levels of ACE2 and TMPRSS2 were examined in trophoblast cells isolated from the placenta of the PE and normotensive group. Both markers showed lower mRNA expression in the PE group; however, there was no significant difference in the protein expression of TMPRSS2 between the PE group and the normotensive control group.

Since the first detected COVID-19 outbreak in 2019, maternal morbidity and mortality during pregnancy have increased; not only respiratory but also non-respiratory pregnancy-related complications of SARS-CoV-2 infection have been observed. In several observational studies, it was reported that pregnant women affected by COVID-19 infection had a higher incidence of hypertensive disorders during pregnancy (18-20). Therefore, a causal relationship between hypertensive disorders in pregnancy and SARS-CoV-2 infection has been suggested (12). However, it has been reported that hypertensive disorders in pregnancy due to infection with COVID-19 can lead to development of a PE-like syndrome rather than true PE with placental complications (11). In addition, Hecht *et al.* found that SARS-CoV-2 infection of the placenta was not associated with a specific placental pathology (13). Placenta-associated 'true' PE can result in a poor perinatal outcome, including preterm birth, considering that termination of pregnancy is accepted as a definitive cure for PE. Complications caused by SARS-CoV-2 infection should be differentiated because a PE-like syndrome alone is not an obstetric indication for delivery. It is necessary to differentiate between the two because a hasty delivery can result in an unfavorable

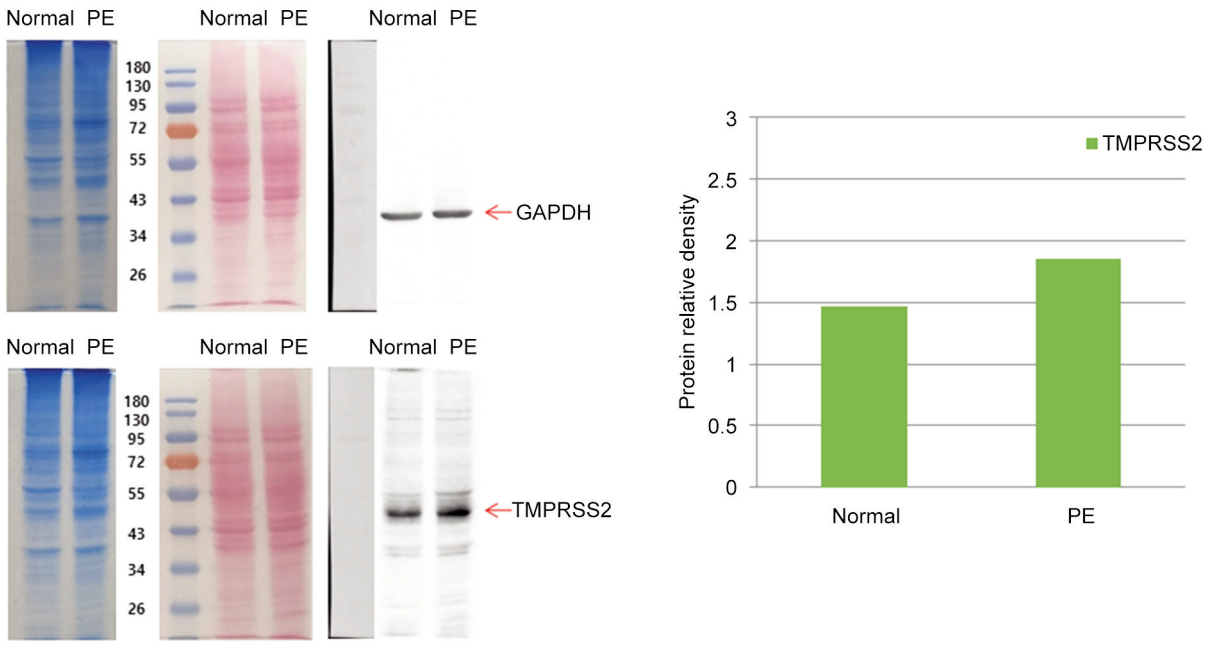

Figure 4. *TMPRSS2 protein expression evaluation in human placental trophoblast cells, using western blotting. TMPRSS2 protein expression did not present any significant difference between PE trophoblast cells and normal controls. PE: Preeclampsia.*

perinatal outcome. Therefore, a novel diagnostic biomarker is needed to discriminate actual PE patients from those with COVID-19 infection.

ACE2 exists in two forms: i) attached to the membrane of cells: mACE2; ii) in a soluble form: sACE2. Soluble ACE2 catalyzes angiotensin II hydrolysis to form angiotensin, which leads to localized vasodilation and lowers the blood pressure. On the other hand, mACE2 facilitates the entry of some coronaviruses, including HCOV-NL63, SARS-CoV, and SARS-CoV-2. The SARS-CoV-2 spike protein causes the down-regulation of sACE2, which damages vascular ECs. A decrease in sACE2 can affect normal blood pressure levels regulated by the renin-angiotensin system in the feto-maternal circulatory system, causing hypertensive diseases such as PE (9). Hypothetically, during COVID-19 infection, the balance of the renin-angiotensin system axis is disrupted, which can lead to a much worse prognosis for both mothers and babies if PE mothers are infected. One of the morphological changes in the placenta observed in PE is increased fibrin deposition (17, 21). In our study, the association of ACE2 expression in placental tissue with PE was not significant; however, a significant relationship was identified with fibrin deposition. Before the COVID-19 outbreak, a study showed that the expression of *ACE2* mRNA was reduced in the placenta of patients with PE complicated with fetal growth restriction (22). The results of that study are in line with our work, which showed low mRNA expression of ACE2 in PE placental trophoblast cells (22).

Some coronaviruses, including SARS-CoV-2, are activated by TMPRSS2 located in the cell membrane, leading to the cleavage of the SARS-CoV-2 spike protein for viral entry into cells (7). TMPRSS2 is known to be upregulated by androgen hormones in prostate cancer and down-regulated in androgen-independent prostate cancer (23). TMPRSS2 expression is increased in asthma and allergic rhinitis. In particular, in asthma, TMPRSS2 expression is positively correlated with type 2 cytokine-induced genes, including CLCA1 and SERPINB2 (10). ACE2 is an attachment receptor for SARS-CoV-2 entry, and the spike protein of SARS-CoV-2 is primed by TMPRSS2. Therefore, the inhibition of ACE2 and TMPRSS2 may provide a therapeutic option against SARS-CoV-2 infection (7). To the best of our knowledge, limited studies have reported the expression of TMPRSS in the placenta except for its association with coronavirus entry. In our study, low nuclear TMPRSS2 expression in ECs was associated with PE, which showed a significant correlation with higher systolic blood pressure and higher urine protein-to-creatinine ratio.

Although the exact mechanism or cause of PE has not been identified, the myometrial component of vascular conversion can fail in PE in a trophoblast-independent and/or trophoblast-dependent manner (24, 25). Prior to trophoblast invasion, the trophoblast-independent mechanism, also termed decidual-associated transformation (26), is known to involve the infiltration of decidual NK cells residing in the

vascular smooth muscle, leading to EC and muscular cell apoptosis. In this study, differences in TMPRSS2 expression in placental ECs and FBs but not trophoblasts were found to be associated with PE. The lack of difference in the expression of the TMPRSS2 protein in PE and normotensive trophoblast cells may also indicate that TMPRSS2 is involved in a trophoblast-independent mechanism. Furthermore, the mRNA expression of TMPRSS was low in PE trophoblast cells; however, there was no difference in the expression of the protein despite the low gene copy number of TMPRSS2, suggesting that a positive feedback mechanism physiologically maintained the appropriate expression levels in the PE group.

To the best of our knowledge, this is the first study to evaluate COVID-19-related protein expression of TMPRSS2 and ACE2 in PE placental tissue and trophoblast cells investigating the effectiveness of these markers as a diagnostic tool for PE. The results of the study suggest that examining TMPRSS2 expression in placental tissue ECs and FBs is helpful for differentiating between clinically indistinguishable PE and a PE-like syndrome caused by SARS-CoV-2 infection. Our findings could contribute to improving the perinatal outcome of pregnancy-related hypertensive disorders during the COVID-19 pandemic. This study had certain limitations. First, as the placental tissue was used, it cannot support clinical decision making before the delivery. However, it can be helpful for postpartum maternal care depending on TMPRSS2 expression in the placenta. Second, maternal SARS-CoV-2 infection could be confirmed at the time of childbirth; however, it was not possible to determine whether there was a history of SARS-CoV-2 infection including asymptomatic infection during pregnancy. Considering this, we suggest that pathological analysis of nuclear TMPRSS2 expression in the ECs in the placenta even after the delivery might be helpful in differentiating true PE from PE-like syndromes. Although most of the pathological conditions and symptoms of PE resolve after birth, PE itself is an official risk factor for cardiovascular disease (27, 28), and there is a risk of recurrence of PE in the subsequent pregnancy. Therefore, patient education as precautions and preventive measures are important, and for this, an obvious diagnosis must be established. The study suggests an association of ACE2 and TMPRSS2 with PE. In addition, TMPRSS2 may be related to a trophoblast-independent PE mechanism and thus may be a novel diagnostic tool to distinguish true PE from complications due to SARS-CoV-2 infection. More in-depth studies using ACE2 and TMPRSS2 will be helpful to elucidate the mechanisms of PE.

Conflicts of Interest

The Authors declare that there are no conflicts of interest.

Authors' Contributions

JEP, HJA: Conception, design, data acquisition and interpretation, and manuscript drafting and editing; DHS, YMK: data acquisition, interpretation, and drafting of the manuscript; HCJ, JCB, HK, JY data acquisition and analysis. All Authors approved the manuscript.

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